

### Colony PCR: (GoTaq Protocol)

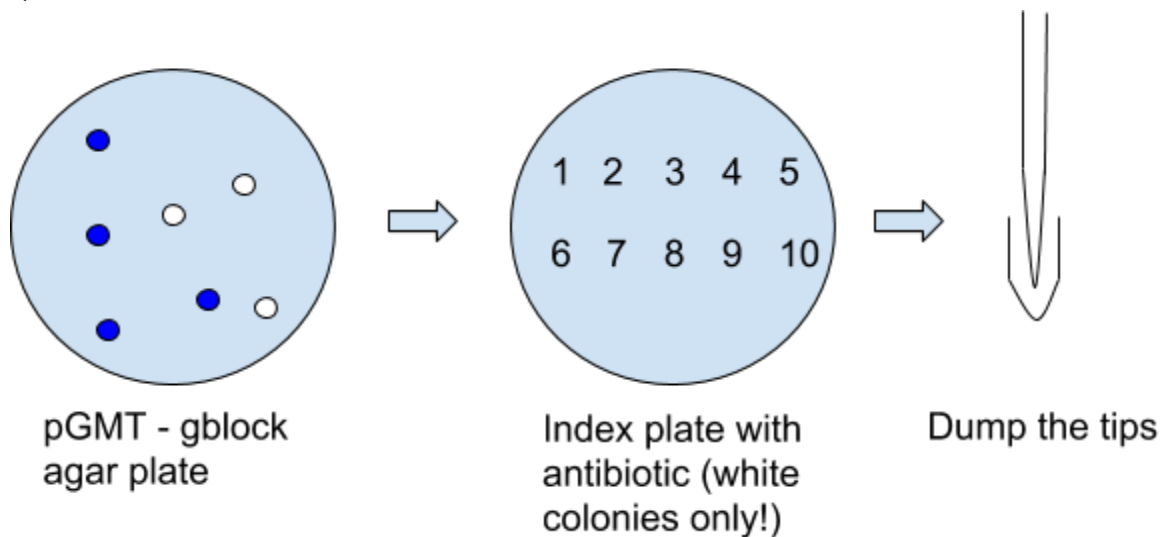
#### 1) Mastermix:

- Primer F: .5µl
- Primer R: .5µl
- dNTP: .5µl
- Colorless 5X buffer: 5µl
- Taq: .125 µl
- dH2O: 18.5 µl
- Total: 25 µl X N

$$N = (\# \text{ of colonies} + 1 \text{ positive control} + 1 \text{ negative control}) \times 1.1$$

#### 3) Vortex

#### 4) Index Plate with antibiotic



#### 5) Put them in thermocycler

	T (C°)	Time	Cycle
Initial Denaturation	95	2 min	1
Denaturation	95	30 s	35
Annealing	60	30 s	
Extension	72	<b>1 min/kb</b>	
Final Extension	72	5 min	1
hold	4	Infinity	1

6) Put your plate in 37 °C incubator

Tips:

- Use at least 10 colonies
- Negative control: leave out template
- Index pGEM with 10 colonies